"Studies on investigation of the biological effects of selected medicinal plant extracts on gram Pod borer - *Helicoverpa armigera"*

A

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By

Pramod S. Deshmukh

Under the Guidance of

Dr. Sudhakar Shamrao Ingale

Professor
Department of Botany,
Narayanrao Waghmare Mahavidyalaya behind irrigation camp, Akhada Balapur Dist Hingoli.

At

Botany Research Centre. N.E.S. Science College, Nanded

1.Introduction :-

Chickpea (*Cicer arietinum* L.) is an essential annual pulse crop that belongs to the genus *Cicer* (Family: Leguminosae, Fabaceae) and is also recognized as "Garbanzo bean" or "Bengal gram" [1]. It is the third-largest food legume produced worldwide, after *Pisum sativum* L. (field pea) and *Phaseolus vulgaris* L. (common bean) [2,3]

Chickpea (Cicer arietinum L.), commonly known as Bengal gram, gram or chana, originated from South Western Asia, is an important rabi pulse crop of India, which has been considered as king of Pulses [4]. Chickpea was cultivated in an area of 135.4 lakh hectares with a production of 131.02 lakh tones and a productivity of 968 kg/ha in the world

This crop is cultivated in about 101 Lha in india. The country harvested a record production of 116 Lt at a highest productivity level of 1145 kg/ha. As usual, Madhya Pradesh has contributed a significant 25% of the total gram area and 30% of total gram production in the country, thereby ranking first both in area and production followed by Rajasthan (20% & 19%), Maharashtra (21% and 19%), and Gujarat (6% & 8%). About 98 per cent of gram production of the country during the period under report has been realized by 10 states of Madhya Pradesh, Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, Karnataka, Andhra Pradesh, Jharkhand, Chhattisgarh and Telangana [5]

Looking into the increasing population, it is expected that by 2050 the domestic requirement for chickpea will increase from 0.4 million to 0.7 million in Africa and from 7 million to 14 million in Asia [6,7].

Helicoverpa armigera is one of the serious pests of chickpea, which feeds more than 150 crops throughout the world [8]. Gram pod borer is widely distributed and a serious pest of chickpea causing Helicoverpa armigera is the major and most devastating pest of chickpea which can cause crop loss up to 80 per cent under congenial weather conditions.

Identification of the pest

- Eggs – are spherical in shape and creamy white in colour, laid singly
- Pupa – brown in colour, occurs in soil, leaf, pod and crop debris
- Adult - light pale brownish yellow stout moth.
- Forewing grey to pale brown with V shaped speck.
- Hind wings are pale smoky white with a broad blackish outer margin.

Symptoms of damage

- Skeletinization of leaves – feeding chlorophyll only leaving veins by young larvae Defoliation
- Feeds flower and green pods
- In green pods – make circular holes and feed the grains and make empty.

Present control methods:- Conventionally farmers are using various types of synthetic chemical insecticides to control gram pod borer. But the unconscious and unjustified use of synthetic pesticides creates several problems in agro ecosystem such as direct toxicity to beneficial insects and humans. The repeated use of systemic insecticides alone has resulted in the development of resistance in the insect pest, and disturbance to the agro-ecosystem by affecting the non-target ones. Farmers largely follow the chemical method as it gives quick results. High frequency application is the common scenario. However these chemicals in many cases invited the problems of pesticide resistance, resurgence, secondary pest outbreak, environmental contamination, residual toxicity, phytotoxicity and toxicity to beneficial organisms like predator and parasitoids as well as disturbance in homeostasis of natural population (Patil et al., 2018) [9]

Bt is a facultative, aerobic, gram-positive and bacterium-forming endospore. It produces a wide range of virulence factors, both during the vegetative and the stationary phases, which contributes to its insecticidal activity. Crystalline toxins or δ-endotoxins are considered the main factor conferring entomopathogenic properties to this bacterium. Despite extensive research for the development of *Bt*-based bio-insecticides, many formulations were ineffective under field conditions because of abiotic stress. To overcome these problems, several key areas/axes have to be assessed to develop an efficient bio-insecticide: spectrum of action, persistence, biodegradability and efficacy. Thus, delivery systems using inexpensive adjuvants or additives have been developed and tested under field conditions [28]

The bacterium *Bacillus thuringiensis* (*Bt*) produces delta-endotoxins that possess toxic properties and can be used as biopesticides, as well as a source of genes for the construction of transgenic plants resistant to insects. In Brazil, the introduction of *Bt* soybean with insecticidal properties to the velvetbean caterpillar, the main insect pest of soybean, has been seen a promising tool in the management of these agroecosystems. However, the increase in stink bug populations in this culture, in various regions of the country, which are not susceptible to the existing genetically modified plants, requires application of chemicals that damage the environment. [29]

Management

- **ETL:** 2 early instar larvae/plant 5-8 eggs/plant
- Pheromone traps for *Helicoverpa armigera* 12/ha
- Bird perches 50/ha
- Hand picking of grown up larvae and blister beetles

- Ha NPV 1.5 x1012 POB/ha with teepol (1 ml/lit.)

Apply any one of the following (Spray fluid 625 ml/ha)

- Dichlorvos 76 WSC 625 ml/ha
- Neem seed kernel extract 5% (31.0 kg/ha) twice followed by
- Triazophos 40 EC 780 ml/ha
- Neem oil 12.5 lit./ha
- Phosalone 35 EC 1.25 lit./ha

On Farm Trials during rabi2017-18 and rabi2018-19 at farmer's field to assess the efficacy of insecticides for the management of Gram Pod Borer (Helicoverpa armigera) in Gram. On the basis of three treatments assessed i.e. (a) Farmer's practice (No Spray) (b) Cypermethrin 25EC @1.5ml./lt. of water(recommended) (c) Indoxacarb 14.5Sc @1ml. /lt. of water(Intervention).The results of OFT during 2017-18 revealed that use of Indoxacarb 14.5Sc @ 1ml. /lt. of water was observed to be superior resulting in 74.46% reduction in damage to pods of Gram and increased the grain yield to an extent of 47.36% over farmers practice (No spray) followed by Cypermethrin 25EC @1.5ml./lt. of water resulting in 67.69% reduction in damage to pods of Gram and increased the grain yield to an extent of 34.21% over farmers practice (No spray) and the results of OFT during 2018-19 revealed that use of Indoxacarb 14.5Sc @ 1ml. /lt. of water was observed to be superior resulting in 71.42% reduction in damage to pods of Gram and increased the grain yield to an extent of 55.55% over farmers practice (No spray) followed by Cypermethrin 25EC @1.5ml./lt. of water resulting in 61.03% reduction in damage to pods of Gram and increased the grain yield to an extent of 38.88% over farmers practice (No spray).[21]

Resistance developed to chemical control:-

Thus, we need to use integrated approaches for the control of gram pod borer in order to avoid indiscriminate use of pesticides. Therefore, it is now an urgent need to use integrated approaches for the control of gram pod borer in order to avoid indiscriminate use of pesticide.

Medicinal plant extracts of *Jatropa curcas, D. metel*

Plants like mentha, jatropa shows inhibitory effect to control the pod borer we will be checking the effects of these natural ingredients on chick pea to control pod borer and their action of mechanism on the different developmental stages of pod borer for effective control.

Euphorbs such as *Jatropha* are a diverse group of plants that are biochemically rich in fatty acids, alkaloids, and terpenoids. Upon a tree's harvest, these compounds could be extracted as new and lucrative sources of biopesticides. *Jatropha* plants chemically defend their tissues with unpalatable or acutely poisonous compounds in the form of a milky sap or 'latex'. This toxic sap resides in tubules that, when ruptured by feeding insects, sicken or kill the. Currently, 170+ described species of *Jatropha* occur throughout the Neotropics. Their ancient origin precedes the breakup of Gondwana, providing some 85 million years for the evolution of a formidable chemical defense. In the last few centuries, *Jatropha* plants and their insect-lethal toxins have served as useful sources of medicine. The genus name *Jatropha* is of Greek origin and literally

translates to ‘doctor’s food’, no doubt a moniker befitting these plants’ use as a common source of early folk remedies.[22]

Several reviews have been conducted on the different species of the genus *Jatropha* covering various aspects such as their ethnobotany, medicinal properties, phytochemistry, and toxicity among others. Phytochemical studies of the genus *Jatropha* have increased in recent years due to the high potential of these species as natural sources of bioactive compounds. Investigations of the chemical constituents of *Jatropha* plants resulted in the isolation of a number of alkaloids, cyclic peptides, terpenes (monoterpene, sesquiterpenes, diterpenes and triterpenes), flavonoids, lignans, coumarins, coumarino-lignoids, a non-cyanogenic glucoside, phloroglucinols, ester ferulates, phenolics, deoxypreussomerins and fatty acids. Moreover, extracts and isolated compounds from various species of this genus have been found to possess properties of cytotoxicity, antimicrobial, anti-inflammatory, antioxidant, insecticidal, larvicidal, cholinesterase inhibition, and toxicity activities [26].

Under the Solanaceae family, there is evidence of 9–14 species of the Datura genus, but not every species has significant distinction to identify it, so only 9 species are widely acceptable; those are *D. metel* *D. ceratocaula* Ortega, *D. discolor* Bernh, *D. ferox* L., *D. innoxia* Mil, *D. kymatocarpa* Barclay, *D. leichhardtii* Benth, *D. metel* L., *D. quercifolia* Kunth, *D. stramonium* L., and *D. wrightii* Regel. is available in Asian and African region

D. metel is a perennial plant. The structure of leaves is simple: alternate, dark green, shallowly lobed, and broadly ovate. The appearance of flowers is large, single, and trumpet- growth, extending branches, and an herbaceous plant of about 1.5 m height. The structure of leaves is simple: alternate, dark green, shallowly lobed, and broadly ovate. The appearance of flowers is large, single, and trumpet-shaped, with a pleasant fragrance and various colors; for instance, in some species, they are white to yellow, and in others, they are light to dark purple. The fruit is egg-shaped, capsulated with short spines, and its diameter is 5 cm.

D. metel contains a lot of significant phytochemicals, including alkaloids, flavonoids, tannins, phenols, cardiac glycosides, amino acids, and carbohydrates. Various parts of the herb are being utilized as hallucinogenic, narcotic, anti-tussive, antispasmodic, bronchodilator, and anti-asthmatic and are used in epilepsy, diarrhea, hysteria, skin diseases, rheumatic pains, painful menstruation, hemorrhoids, wounds, burns, and skin ulcers. *D. metel* is considered an astringent, bitter, germicide, acrid, anti-phlogistic, anodyne, narcotic, antiseptic, and sedative medicinal plant in Ayurveda (the Ayurvedic Pharmacopoeia of India). Haploid embryos were also developed by Anther culture to produce a haploid *D. metel* plant [25]

.Structure-activity relationships (SAR) are key to many aspects of drug discovery, ranging from primary screening to lead optimization. Working with SAR starts from identifying whether an SAR actually exists in a collection of molecules and their associated activities to trying to elucidate the details of one or more SARs and subsequently using that information to make structural modifications to optimize some property or activity. Fundamentally, an understanding

of the SAR for a set of molecules, allows one to rationally explore chemical space, which, in the absence of "sign posts" is essentially infinite [16].

SAR depends on the recognition of which structural characteristics correlate with chemical and biological reactivity. Thus the ability to draw conclusions about an unknown compound depends upon both the structural features that can be characterized as well as the database of molecules against which they are compared.

When combined with appropriate professional judgment, SAR can be a powerful method for understanding functional implications when similarities are found. For example, in the case of risk assessment of uncharacterized compounds, data from the most sensitive toxicological endpoints should be included in the analysis, such as carcinogenicity or cardio toxicity. [17]

QSAR analysis is particularly useful during the discovery stage for selection of chemicals for further development. QSAR also can be used for prioritization of chemicals for various actions related to health and safety and environmental assessment. The elements generally needed for QSAR include a verified bioassays database for the end point to be predicted, a set of chemical–physical parameters which described the chemical structures so that the end point can be modeled in terms of these parameters, statistical techniques, that is, principally multivariate regression and discriminant analysis for weighing these parameters in a near-optimum fashion for the explanation of the end point, and computer technology to make it all practical.

QSAR-Co: An Open Source Software for Developing Robust Multitasking or Multitarget Classification-Based QSAR Models [28]

2. Literature review:-

Kabuli and desi are the two types of cultivated chickpea where 80–85% of the chickpea area is represented by desi type [1]. Desi type is usually grown in semi-arid tropics and Kabuli in the temperate region. The seed's size, shape, and color are used to categorize it. Desi seeds are small, colored, and angular in shape, but kabuli seeds are huge, shaped like an owl's head, and beige in color [10]

Chickpea plant grows quickly, branch, and attains a height of 20 to 60 cm, or even 1 m. It contains multiple secondary lateral roots that delve into the top soil layers in addition to a large taproot that can reach a depth of two meters (15–30 cm). The leaves are five cm long and have ten to twenty sessile, oblong to elliptic leaflets. The stems are hairy, straight or twisted, simple or branching. The single, papilionaceous flowers are pink, white, blue, or purple in color [11,12].

On average, 50 to 60 pods grow. The pods are pubescent, inflated, and oblong. The seeds (usually one seed per pod) come in a range of colors, shapes, and sizes (creamy-white to black; spherical to angular; 5 to 10 mm in diameter). In India, it is usually grown in a cool season

rainfed in semi-arid regions, or as a dry climate crop. It requires an optimum temperature of around 21–29 °C and 18–26 °C at night whereas 600–1000 mm of annual rainfall [13,14]. In many tropical regions, the plant is effectively cultivated in the cool season under irrigation and is well adapted to fairly temperate tropical countries. Chickpea plants are often planted in deep red or black soils (pH of 5.5–8.6) [15].
The chickpea crop is attacked by a number of insect- pests from seedling to its maturity. The major insect- pests attacking chickpea crop are Helicoverpa armigera, Spodoptera litura, Agrotis ipsilon, Plusia orichalchea and Bemisia tabaci during winter and summer seasons. The chickpea pod borer, Helicoverpa armigera is polyphagous in nature which causes damage to several crops such as pigeon-pea, groundnut, cotton, vegetables, pearl millets, sorghum, maize, sunflower etc. The young larvae often feed upon the tender foliage before attacking the pods by causing heavy losses to crop and sometimes whole crop failed due to severe infestation [11,12]

Use of chemical pesticides has resulted in immediate high returns to farmers. However, their heavy and extensive use has created various health and environmental problems. To avoid these problems, use of environmentally safer bio-insticides is gaining momentum these days

Jatropha is a genus of flowering plants in the spurge family, Euphorbiaceae. The name is derived from the Greek words ἰατρός (*iatros*), meaning "physician", and τροφή (*trophe*), meaning "nutrition", hence the common name **physic nut**. Another common name is **nettlespurge**. It contains approximately 170 species of succulent plants, shrubs and trees (some are deciduous, like *Jatropha curcas*). Most of these are native to the Americas, with 66 species found in the Old World.[3] Plants produce separate male and female flowers. As with many members of the family Euphorbiaceae, *Jatropha* contains compounds that are highly toxic. *Jatropha* species have traditionally been used in basketmaking, tanning and dye production. In the 2000s, one species, *Jatropha curcas*, generated interest as an oil crop for biodiesel production and also medicinal importance when used as lamp oil; native Mexicans in the Veracruz area developed by selective breeding a *Jatropha curcas* variant lacking the toxic compounds, yielding a better income when used as source for biodiesel, because of its edible byproduct. Toxicity may return if edible *Jatropha* is pollinated by toxic types.[24]

Jatropha curcas L. is a Euphorbiaceae widely distributed in tropical and subtropical regions, characterized by rusticity, resistance to drought and low susceptibility to pest attack. The latter characteristic can be attributed to the presence of compounds toxic to animals, such as lectins, saponins, phytates, trypsin inhibitors and phorbol esters. These compounds can be found in the whole *J. curcas* plant, but are more abundant in its seeds. Studies report that seeds of *J. curcas* present nematicide, fungicide and molluscicide activity[23].

India has a great wealth of various naturally occurring plant drugs which have great potential pharmacological activities. *Datura stramonium* (*D. stramonium*) is one of the widely well known folklore medicinal herbs. The troublesome weed, *D. stramonium* is a plant with both poisonous and medicinal properties and has been proven to have great pharmacological potential with a

great utility and usage in folklore medicine. *D. stromonium* has been scientifically proven to contain alkaloids, tannins, carbohydrates and proteins.

Parts Of Datura Used In Medicinal Formulations

Datura plant as a whole has several characteristic properties including anti-spasmodic, analgesic, sleep-inducing, expectorant, sedative, hypnotic, intoxicant, uterine stimulant and bronchodilator properties.

Owing to its several therapeutic properties, the entire plant, be it the fresh or dry leaves, seeds, roots, or fruits, Datura has carved its way in many traditional and alternative medications.

Datura seeds when used in raw form are extremely dangerous and poisonous, hence before using them in any formulation, the seeds require a series of sodhana or purification process, to detoxify the harmful effects, so as to reduce the toxicity levels in the human body and to promote the therapeutic value of the part.

Pharmacological Constituents Of Datura

Imbibed with powerful hallucinogenic and deliriant properties, Datura has a mother lode of antioxidants, flavonoids, alkaloids, organic compounds, and minerals. The bioactive components in Datura include Daturine, Daturadiol, Hyoscine, Atropine, Noratropine, Fastudine, Allantoin, Hypocyamine, Norhyosciamine, Tropane, Meteolodine, Scopolamine, Mucilage, Albumen, Vitamin C, Niacin, and Malic Acids.[27]

B. thuringiensis can also serve as a source of toxic genes that can be expressed in plants and thus confer toxic property against different species of insect pests. Genetically modified plants (GMPs) that express the *Bt* genes, such as rice, corn, potato, cotton, and soybean are associated with the control of pests, especially Lepidoptera. The resistant cultivars result in increased productivity, greater economic value, reduction in the use of chemical pesticides, and benefits in the selectivity of the target pest. Moreover, *Bt* plants favor the exposure of target insects throughout infestation period, including pests in their most vulnerable stages of development. In another instance, the specificity of toxins of this bacterium is effective for only a limited number of species and the development of resistance to modified plants is more specific than other insecticides .[30]

While it is certainly true that computational methods can help in identifying, explaining and predicting structure-activity relationships (SAR), it is also true that naïve usage (or even misuse) of these techniques can lead to misleading results. Fundamentally, SAR models are just that: models. That is, a reduced or simplified representation of reality, replete with assumptions and limitations. These methods cover the spectrum in terms of complexity and utility. The goal of this chapter is to highlight the different type of SAR modeling methods, and specifically, how they support the task of exploring chemical space to elucidate and optimize structure-activity relationships in a drug discovery setting. In addition to considering modeling algorithms, I will also briefly discuss the use of databases as a source of SAR data and how they can be used to inform and enhance the exploration of SAR trends.

Quantitative structure–activity relationships (QSAR) modeling is a well-known computational technique with wide applications in fields such as drug design, toxicity predictions, nanomaterials, etc.

SARANEA: a freely available program to mine structure-activity and structure-selectivity relationship information in compound data sets

3) Objectives:-

a. To Collect, identify and authenticate selected medicinal plant.

b. To Screen effect of the selected plant extracts against different developmental stages of pod borer.

c. To standardize the optimum condition for experimental studies.

d. To Evaluate the effect of the medicinal plants extract on pod borer and combinations of extracts.

e. To analyzing SAR results of for desirable binding of plant biomolecules with the target protein / enzymes of the insect.

4. Materials Methodology:-

a) Tools for plant part collection from selected medicinal plants *datura melitus* and ***Jatropha curcas*** b) apparatus like soxlet apparatus for separation of medicinal ingrediants c) software, tools for structure activity relationship – SARANEA, QSAR-Co

Soxhlet extraction: Soxhlet extraction is also known as continuous hot extraction. The apparatus used is known as Soxhlet extractor, which consists of a round bottom flask, extraction chamber, extraction thimble, siphon tube, and condenser. The finely powdered plant material is placed inside a porous bag (thimble) and this thimble is placed in extraction chamber. The extraction solvent placed in the round bottom flask is continuously heated and its vapours condense in a condenser and drip into the plant material in the thimble and extracts it by contact. When the level of the solvent in thimble reaches the top of siphon tube, the contents in the extraction chamber (extracted material and the solvent) siphon into the round bottom flask. It is a most common method used for plant extraction, as the process consumes less time and solvent for extraction, and no filtration is required.[31]

phytoconstituents	Polarity	Solvents used for extraction
Alkaloids	polar	Methanol, ethanol
Flavonoids	Polar	Methanol, ethanol,water
Polyphenols	Highly Polar	Methanol, ethanol,water
Tannins	Moderate/non-polar	Diethyl ether,hexane,chloroform
Terpenoids	Non-polar	Hexane,chloroform,petroleum ether, toluene
Glycosides	Polar	Water,methanol,ethanol
Anthocynins	Polar	Water,methanol,ethanol
Coumarins	Polar	Water,methanol,ethanol
Anthraquinones	Non-polar	Hexane,chloroform,petroleum ether,toluene
Steroids	Highly non-Polar	Hexane,toluene,petroleum ether

Microwave-assisted extraction: It is one of the advanced techniques which uses mechanism of dipole rotation and ionic transfer by ion displacement in the solvent and the plant material. This method is mostly used to extract polar compounds. To the plant material dissolved in solvent, electromagnetic radiation (300 MHz to 300 GHz) is applied, which got converted into heat when absorbed by the plant material-solvent mixture resulting into the dipole rotation and ion migration in the solvent which facilitates solvent penetration and extraction process. However, this process is not useful when nonpolar solvent is used as a very small heat is produced due to low dipole movement. This technique uses less solvent and time for extraction .[31]

Maceration: Maceration is a process that entails soaking plants in water. The plant material is soaked for some time, after which it is filtered out from the plant extract-laden water. Subsequently, the collected liquid is heated and cooled. This process is repeated until all required compounds are extracted from the raw materials.

Decoction or reflux: Decoction is an extraction technique by boiling the active substances present in herbs and other plants (stems, roots, bark, etc.).

Digestion: Digestion is a hot maceration; in other words, the contact phase is maintained at a lower temperature than its boiling point.

Infusion: the boiling solvent is poured onto the solid and then left to cool for a given time.

Leaching or percolation: During the plant percolation, the solvent passes slowly through the biomass to extract the soluble compounds of the plant.

6. Proposed Work Plan /Formulation and Structure of study:

First Year:-

(a) Reference work study

(b) Survey, Collection and Identification of plants of jatropa and datura.

(c) Separation and processing of required plant part.

Second Year:-

(a) Growing pod borer at its different developmental stages egg,pupa,adult,larva

(b) Purification of biologically active compounds from various plant parts.

(c) Assesment of toxic properties of the plant extract against pod borer

Third Year:-

(a) Characterization and purification of the compound.

(b) Assessment and application of the purified extract components for its activity.

(c) Checking SAR(structure activity relationship of active compounds with stem borer life critical enzymes).computer based step.

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Mr. Pramod S. Deshmukh
Research Scholar

Dr. Sudhakar S. Ingale
Research Guide